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I, ANNA MAIJA MADL, ACTING TEAM LEADER EXAMINATION SUPPORT & SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PP 7916 for a patent by UNIVERSITY OF SYDNEY filed on 23 December 1998.

I further certify that the name of the applicant on the cover page of the specification has been amended from THE UNIVERSITY OF SYDNEY to UNIVERSITY OF SYDNEY pursuant to the provisions of Section 104 of the Patents Act 1990.

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Sixteenth day of February 2000

A. M. Madl.

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PROVISIONAL SPECIFICATION

for the invention entitled:

"An assay"

The invention is described in the following statement:

of oncogenes and tumour suppressor genes. Furthermore, disease conditions or disorders associated with changes in cell cycle and development can be attributed to changes in transcriptional regulation of particular genes.

Although there are a number of genetic assays available to assess mutations, the identification of certain genetic changes cannot always be directly indicative of a disease condition or disorder.

Some genetic changes are expressed by alterations in cell surface antigens. Again, however, prior attempts to develop a diagnostic assay for complex disease conditions or disorders such as cancer based on the identification of a single antigen have not been universally successful.

Leukemias and lymphomas cause significant mortality and morbidity in humans. Such cancers result from the continuous proliferation of cells which would otherwise be blocked at various stages of normal differentiation to specialised cell types. Leukemias arise from blood forming cells in the bone marrow due to mutations in any of the precursors in the various lineages of differentiation (see Figure 1). Lymphomas develop from lymphocytes or macrophages in lymphatic tissue.

Lymphocytes in the peripheral blood express a large number of different antigens on their outer plasma membranes which are receptors for growth factors, cell-cell interactions and immunoglobulins; molecules for cell adhesion or complement stimulation; enzymes and ion channels. A single systematic nomenclature has been developed to classify monoclonal antibodies against human leukocyte cell surface antigens known as the cluster of differentiation (CD) antigens (Schlossman *et al,* 1995). Detailed information on CD antigens can be found at http://www.ncbi.nlm.nih.gov/prow/cd/index_molecules.htm. The expression of these cell-surface antigens can distinguish different types of mature blood cells found in the peripheral circulation.

Cells in the peripheral blood are produced in the bone marrow by proliferation and differentiation down specific lineages from precursor myeloid or lymphoid stem cells which

resulting pattern of antigen expression is then indicative of the disease condition or disorder or a propensity for development of a disease or disorder.

SUMMARY OF THE INVENTION

One aspect of the present invention provides a diagnostic assay device comprising an array of molecules wherein in each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the pattern of interaction between the molecules and the binding partners is indicative of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

Another aspect of the present invention contemplates an assay device for the diagnosis of cancer or a propensity for the development of cancer in an animal such as a human, said assay device comprising an array of molecules immobilized to a solid support wherein each molecule of the array, with the exception of a negative control, is capable of interaction with a respective binding partner if present in a biological sample from said animal wherein the pattern of interaction between the immobilized molecules and their respective binding partners is indicative of the presence of cancer or a propensity to develop cancer.

A further aspect of the present invention is directed to an array of molecules immobilized on a solid support said array defined by the formula:

$$\left[\left[Px_1\right]^{n_1}_{b} \left[Px_2\right]^{n_2}_{c} \ \ldots\ldots \ \left[Px_j\right]^{n_i}_{d} \right]_z$$

wherein

P is a member of a binding group capable of interacting with a binding partner;

n_1 n_2 ..... n_i represent different members of the binding group;

x_1 x_2 ..... x_j represent different binding groups;

m_1 m_2 ..... m_l represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the number of groups of immunoglobulins on the array and is from about 1 to about 2000;

wherein the pattern of interaction between the immobilized immunoglobulins and their respective antigens is indicative of the development of cancer or a propensity to develop cancer.

Yet a further aspect of the present invention contemplates an assay device for cancer said device comprising an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different cluster of differentiation antigens and/or myeloid antigens expressed on leukemic cells wherein the binding pattern of the immobilized immunoglobulins to their respective antigens is indicative of the presence of cancer or a propensity to develop cancer.

Another aspect of the present invention provides a diagnostic assay device comprising an array of molecules wherein in each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the molecules are in an arrangement in said array such that upon interaction between the molecules and the binding partners a differential pattern of density provides an identifiable signal which is indicative of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

A further aspect of the present invention contemplates an assay device for the diagnosis of cancer or a propensity for the development of cancer in an animal such as a human, said assay

comprising an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of normal cells or cancerous cells or are released by normal cells or cancerous cells wherein the binding immunoglobulins are in an arrangement in said array such that the pattern of the immobilized immunoglobulins to their respective antigens provides a differential pattern of density which is in the form of an identifiable signal and is indicative of the presence of cancer or a propensity to develop cancer.

Another further aspect of the present invention is directed to an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1}\right]_{e}^{m_1} \left[q_{o_2}\right]_{f}^{m_2} \cdots\cdots \left[q_{o_k}\right]_{g}^{m_i} \right]_{y}$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 1

thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1}\right]_{e}^{m_1} \left[q_{o_2}\right]_{f}^{m_2} \cdots\cdots \left[q_{o_k}\right]_{g}^{m_i} \right]_{y}$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens;

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 2 to about 2000;

wherein the molecules are in an arrangement in said array such that upon interaction between the immobilized immunoglobulins and their respective antigens a differential pattern of density provides an identifiable signal which is indicative of the development of cancer or a propensity to develop cancer.

Another aspect contemplates a method for determining the presence of a disease condition or disorder or a propensity to develop a disease condition or disorder such as but not limited to cancer in an animal, avian species or plant, said method comprising obtaining a biological sample from said animal, avian species or plant comprising free binding partners or binding

to about 2000;

wherein the pattern of interaction between the immobilized immunoglobulins and their respective antigens is indicative of the development of cancer or a propensity to develop cancer.

Still yet another aspect of the present invention is directed to the use of an array of molecules capable of interaction with a respective binding partner putatively in a biological sample to determine the presence of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

Another aspect of the present invention contemplates a method of treating cancer in a human or non-human animal said method comprising obtaining a biological sample from said human or non-human animal and contacting said sample with an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of normal cells or cancerous cells or are released by normal cells or cancerous cells and determining the binding pattern of the immobilized immunoglobulins to their respective antigens and then undertaking immunotherapy such as with, but not limited to, humanized monoclonal antibodies based on the expression of the antigens.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing lineages for blood cell differentiation with precursor cell types from which various types of leukemias arise. Determination of complete antigen expression would enable unequivocal diagnosis of the leukemia (c.f. Figure 2). CML, Chronic Myeloid Leukemia; AMML, Acute Myelomonocytic Leukemia; ALL, Acute Lymphocytic Leukemia; AEL, Acute Erythrocytic Leukemia; AmegL, Acute Megakaryocytic Leukemia; AMoL, Acute Monocytic Leukemia; AML, Acute Myeloid Leukemia; CLL, Chronic Lymphocytic Leukemia; NHL, Non-Hodgkins Lymphoma; APL, Acute Promyelocytic Leukemia. Adapted from Cooper (1993).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides an assay device comprising an array of molecules capable of interaction with binding partners potentially present in a sample to be tested. The presence or absence of interaction of the assay molecules with binding partners provides an indication of a disease condition or disorder in the animal, avian species or plant from which the sample is derived.

Accordingly, one aspect of the present invention provides a diagnostic assay device comprising an array of molecules wherein each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the pattern of interaction between the molecules and the binding partners is indicative of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

The disease condition or disorder is preferably in an animal such as a human, primate, laboratory test animal (e.g. mouse, rabbit, guinea pig, hamster), companion animal (e.g. dog, cat) or captive wild animal.

The preferred animal is a human.

The present invention also extends, however, to the detection of disease conditions or disorders in avian species and plants. Examples of avian species include poultry birds (e.g. chickens, ducks, turkeys, geese), game birds (e.g. pheasants, wild ducks, peacocks) and flightless birds (e.g. emus, ostriches). Examples of plants include monocotyledonous and dicotyledonous plants including crop plants, fruit trees, ornamental plants and plantation trees.

The disease or disorder most applicable for detection in accordance with the present invention is cancer in animals such as humans. The term "cancer" is used in its broadest sense and includes benign and malignant leukemias, sarcomas and carcinomas. The cancers

nitrocellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid support may be in the form of a membrane or tubes, beads, discs or microplates, or any other surface suitable for conducting an assay. Binding processes to immobilize the molecules are well-known in the art and generally consist of covalently binding (e.g. cross linking) or physically adsorbing the molecules to the solid substrate.

The molecule immobilized to the solid support of the present invention is referred to herein as "P". Molecule p is generally selected from a binding group designated "x". A number, n, of P molecules may be selected from each binding group or one p molecule may be selected from each binding group. The binding groups are selected on the basis of the disease condition or disorder being diagnosed. In the case of cancer, for example, a particular cancer or the development of cancer in general may be determined on the basis of the expression of certain cell-surface ligands (and more particularly antigens) or the release of soluble ligands (e.g. soluble antigens). The P molecules are then selected as binding partners to the cell surface or soluble ligands. The pattern of interaction between the P molecules and their respective binding partners is indicative of the development of, or a propensity to develop, cancer or other disease conditions or disorders.

The array may also comprise a positive and/or negative control to assist in maintaining quality control of the assay procedure. A positive control, for example, may be a P molecule capable of interaction with a binding partner known to be present in the biological sample.

Another aspect of the present invention is directed to an array of molecules immobilized on a solid support said array defined by the formula:

$$\left[\left[Px_1 \right]_b^{n_1} \left[Px_2 \right]_c^{n_2} \ \ldots\ldots \ \left[Px_j \right]_d^{n_i} \right]_z$$

wherein

diagnosis of the disease condition. The array would most preferably further comprise an internal positive and negative control.

Accordingly, another aspect of the present invention contemplates an assay device for cancer said device comprising an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of normal cells or cancerous cells or are released by normal cells or cancerous cells wherein the binding pattern of the immobilized immunoglobulins to their respective antigens is indicative of the presence of cancer or a propensity to develop cancer.

Generally, a range of immunoglobulins is selected on the basis of antigens expressed on normal cells, cancer cells and potential cancer cells. Each group of immunoglobulins specific for a different antigen is defined by $x_1\ x_2\ ....\ x_j$. Generally, a sample would contain a mixed population of cells and the immunoglobulin molecules on the array are designed to selectively bind to antigens on particular cell types.

Within each of groups $x_1\ x_2\ .....\ x_j$, a number of immunoglobulin sub-groups may exist specific for different parts of the same antigen defined by the groups $x_1\ x_2\ .....\ x_j$. These sub-groups are defined by $n_1\ n_2\ .....\ n_i$.

Accordingly, another aspect of the present invention is directed to an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1}\right]_e^{m_1} \left[q_{o_2}\right]_f^{m_2} \ \ldots\ldots\ \left[q_{o_k}\right]_g^{m_i} \right]_y$$

propensity to develop cancer.

Preferably, the immunoglobulins are monoclonal antibodies but the present invention extends to polyclonal antibodies or antigen-binding parts, derivatives, homologues or analogues thereof as well as fusion or hybrid antibodies, synthetic antibodies or recombinant antibodies.

The detection of antigen-immunoglobulin binding may be accomplished in any number of ways. For example, where the antigens are cell surface antigens, the array may be designed to permit the binding of cells to the immobilized immunoglobulins. The captured cells may then be analysed microscopically, with the use of various stains, biochemically or immunologically.

Alternatively, the immobilized immunoglobulins act as capture molecules for particular cell types and the expression of other antigens on normal or cancerous cells is then determined using labelled antibodies or by other convenient means. The capture molecules may also be for soluble antigens or for cells disrupted from solid tumours. In relation to the latter, conveniently, cell suspensions are produced from solid tumour biopsies and the cell suspensions are brought into contact with the immunoglobulin array.

Where the array of immobilized immunoglobulins is designed to bind to free, (i.e. soluble antigen), then the captured antigen may be detected immunologically or by other means.

The present invention is hereinafter described with reference to a particularly preferred embodiment.

The cancer assay device of the present invention is preferably a solid support having a flat, planar surface. Examples of suitable solid supports include membranes, plastic cover slips, glass slides or the wells of microtitre trays. The solid support comprises an array of immunoglobulin coated regions, preferably in the form of spots. The spots are discrete and surrounded by regions not containing any immunoglobulin molecules. The immunoglobulins within a given region or spot are generally immobilized by covalent or non-covalent bonds.

generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to immunoglobulins without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled immunoglobulin adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope.

The presence of cancer or a propensity to develop cancer is indicated by an increase in particular cell numbers or proportion of cell numbers carrying particular antigens.

Another aspect of the present invention contemplates a method for determining the presence of a disease condition or disorder or a propensity to develop a disease condition or disorder such as but not limited to cancer in an animal, avian species or plant, said method comprising obtaining a biological sample from said animal, avian species or plant comprising free binding partners or binding partners bound to a cell surface, said binding parties associated directly or indirectly with said disease condition or disorder and contacting said biological sample with a solid support comprising an array of molecules capable of binding to said binding partners wherein the pattern of interaction with the binding partners is indicative of the disease condition or disorder or a propensity to develop said disease condition or disorder.

Preferably, the binding partners comprise two or more different antigens associated with the disease condition or disorder and the array of molecules is an array of immunoglobulins having specificity for the different groups of antigens.

cancer.

Yet another aspect of the present invention is directed to the use of an array of molecules capable of interaction with a respective binding partner putatively in a biological sample to determine the presence of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

Preferably, the molecules are immunoglobulins.

Preferably, the immunoglobulins are specific for all bound antigens or soluble antigens such as antigens on or shed by cancer cells.

In a particularly preferred embodiment, the pattern of interaction is measured qualitatively or quantitatively as a pattern of density of either cells bound to the molecules in the array or cell-free antigens which have bound to the molecules of the array. The pattern of density may, for example, be determined macroscopically or microscopically or may be made with the aid of artificial intelligence such as using a computer guided densitometer.

Accordingly, another aspect of the present invention provides a diagnostic assay device comprising an array of molecules wherein in each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the molecules are in an arrangement in said array such that upon interaction between the molecules and the binding partners a differential pattern of density provides an identifiable signal which is indicative of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

More particularly, the present invention contemplates an assay device for the diagnosis of cancer or a propensity for the development of cancer in an animal such as a human, said assay device comprising an array of molecules immobilized to a solid support wherein each molecule of the array, with the exception of a negative control, is capable of interaction with

b, c and d represent the number of different members of the binding groups x_1 x_2 ..... x_j; respectively and wherein b, c and d may be the same or different and each is from about 0 to about 100 provided that at least one of b, c or d is not 0;

z is the total number of groups of molecules on the array and is from about 1 to about 2000;

wherein molecules are in an arrangement in said array such that upon interaction between the immobilized molecules and their respective binding partners, a differential pattern of density provides an identifiable signal which is indicative of a disease condition or disorder or a propensity to develop said disease condition or disorder.

Still a further aspect of the present invention contemplates an assay device for cancer said device comprising an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of normal cells or cancerous cells or are released by normal cells or cancerous cells wherein the binding immunoglobulins are in an arrangement in said array such that the pattern of the immobilized immunoglobulins to their respective antigens provides a differential pattern of density which is in the form of an identifiable signal and is indicative of the presence of cancer or a propensity to develop cancer.

Even yet another further aspect of the present invention is directed to an array of immunoglobulins or derivatives or chemical equivalents thereof specific for antigens expressed on normal cells or cancerous cells or released by normal cells or cancerous cells wherein each group of immunoglobulins specific for each antigen or part thereof occupies a discrete region of a solid support, said array defined by the formula:

$$\left[\left[q_{o_1} \right]_e^{m_1} \left[q_{o_2} \right]_f^{m_2} \cdots\cdots \left[q_{o_k} \right]_g^{m_i} \right]_y$$

disease condition or disorder and contacting said biological sample with a solid support comprising an array of molecules capable of binding to said binding partners wherein the molecules are in an arrangement in said array such that upon interaction with the binding partners a differential pattern of density provides an identifiable signal which is indicative of the disease condition or disorder or a propensity to develop said disease condition or disorder.

Yet a further aspect of the present invention contemplates a method of treating cancer in a human or non-human animal said method comprising obtaining a biological sample from said human or non-human animal and contacting said sample with an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of normal cells or cancerous cells or are released by normal cells or cancerous cells and determining the binding pattern of the immobilized immunoglobulins to their respective antigens and then undertaking immunotherapy using antibodies against the expressed antigens. These antibodies are preferably humanized monoclonal antibodies.

The present invention is further described by the following non-limiting Examples.

media. Further fractionation of lymphocyte or other cell sub-populations may be conducted using Dynabeads which are magnetic beads covalently linked to a specific antibody. Generally, cells are resuspended in Hank's solution at a density of 2 x 10^6 cells/ml and are applied to an antibody array as a uniform suspension of 200 μl. The Hanks solution is isotonic and contains glucose which maintains the viability and integrity of the cells during this binding step. After incubation of the cell suspension with the antibody array for 60 minutes at 37°C in a humid atmosphere, the unbound cells are suspended by gentle rocking and the array is subject to washing with pre-warmed Hanks solution (3 x 1 ml). In each case, unbound cells are removed by aspiration of the Hanks solution.

EXAMPLE 3

ASSAY

Samples of cells or antigens are applied to the antibody-membrane array, incubated to enable maximal binding and non-bound material is then removed by a suitable washing solution. Generally, bound cells are fixed to the antibodies by chemical cross-linking. Cells bound to antibodies in the array are visualized microscopically to determine morphologies. Where necessary, cells are fixed to the array and stained, treated enzymically and/or tested for enzymic and/or receptor expression.

Fixed cells may also be interacted with a second antibody labelled with a reporter molecule (e.g. fluorochrome). Alternatively, a series of "second" antibodies are used each with a different fluorochrome. Expression of multiple antigens on cells is then determined by fluorescence confocal microscopy.

Fixed cells are also stained or fluorescently labelled to enable quantification of cell densities in the original body fluid sample. This quantification may be automated with a programmable scanner which records cell densities at each antibody dot in the array. For example, a laser densitometer (Molecular Dynamics) which scans in two dimensions (resolution 60 μm) is particularly useful. The degree of staining of the antibody spots is proportional to the number of cells bound to each antibody. For fluorescence detection, a FluorImager (Molecule

densities of cells expressing a particular surface antigen are quantified by binding cells to a series of uniformly-sized antibody dots which differed in the densities of the same antibody by factors of 10-fold. The density of a particular cell sub-type is determined by densitometric or fluorimetric scanning of fixed and stained cells bound to these serially-diluted antibody dots to determine when the density of antibodies in a particular dot exceeded the density of cells in the sample of cell suspension. A second form of analysis is to run a cell suspension down a strip of a particular antibody linked to a membrane, fix and stain the bound cells, and then measure the length of the stripe of bound cells relative to the length of the total antibody stripe. The density of cells expressing a particular antigen is then calculated from the known binding capacity of antibodies in the stripe. For serially-diluted antibody dots or an antibody stripe, an internal standard is run for an antigen expressed on normal lymphocytes not related to the cancer cells.

Using an array of monoclonal antibodies (e.g. against CD and MY antigens), the pattern of expression of particular antigens identified by this array is matched to set patterns of antigen expression for different leukemias (e.g. M4 AML (acute myeloid leukemia): MY4(CD14), MY7, MY9, MO1(CD11b)). The cellular morphology provides a second criterion for diagnosis. The diagnosis may be automated with fluorometric or spectrophotometric scanning of the arrays to determine which antibody spots bound cells, with computerized recognition of patterns of antigen expression for particular cancers. This method enables automated diagnosis of a wide variety of leukemias, lymphomas and other metastatic cancers. Using a complete array of antibodies against CD and MY antigens, new types of leukemias and lymphomas may be discovered.

Identification of particular antigens on cancer cells provides essential information for subsequent immunotherapy of the patient with humanized monoclonal antibodies, or immunotoxins where a toxin or drug is covalently linked to the antibody. Progressive remission induced by chemotherapy and/or radiotherapy and subsequent relapse due to growth of drug-resistant cells is monitored in patients using periodic cell samples.

Other arrays of antibodies specific for antigens expressed by metastatic colon, breast,

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4. A diagnostic assay device according to aspect 3 wherein the disease condition or disorder is cancer.

5. A diagnostic assay device according to aspect 4 wherein the array comprises immunoglobulins in discrete regions of the solid support and the binding partners are antigens expressed on the surface of a normal or cancerous cell or are released by a normal or cancerous cell.

6. A diagnostic assay device according to aspect 5 wherein the array comprises the formula:

$$\left[\left[q_{o_1} \right]_{e}^{m_1} \left[q_{o_2} \right]_{f}^{m_2} \cdots\cdots \left[q_{o_k} \right]_{g}^{m_i} \right]_{y}$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 2 to about 2000.

7. A diagnostic assay device according to aspect 6 wherein the immunoglobulins are

P is a member of a binding group capable of interacting with a binding partner;

n_1 n_2 ..... n_i represent different members of the binding group;

x_1 x_2 ..... x_j represent different binding groups;

b, c and d represent the number of different members of the binding groups x_1 x_2 ... x_j; respectively and wherein b, c and d may be the same or different and each is from about 0 to about 100 provided at least one of b, c or d is not 0;

z is the total number of groups of molecules on the array and is from about 2 to about 2000.

13. A method of aspect 12 wherein the disease condition or disorder is cancer.

14. A method according to aspect 13 wherein the array comprises immunoglobulins in discrete regions of the solid support and the binding partners are antigens expressed on the surface of a normal or cancerous cell or are released by a normal or cancerous cell.

15. A method according to aspect 14 wherein the array comprises the formula:

$$\left[\left[q_{o_1}\right]_e^{m_1} \left[q_{o_2}\right]_f^{m_2} \cdots\cdots \left[q_{o_k}\right]_g^{m_i} \right]_y$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancerous cell or antigen released by a normal cell or cancerous cell;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided at least one of e, f or g is not 0;

20. Use of an array of molecules capable of interaction with a respective binding partner putatively in a biological sample to determine the presence of a disease condition or disorder or a propensity for the development of a disease condition or disorder.

DATED this 23rd day of DECEMBER, 1998

University of Sydney

By their Patent Attorneys

DAVIES COLLISON CAVE

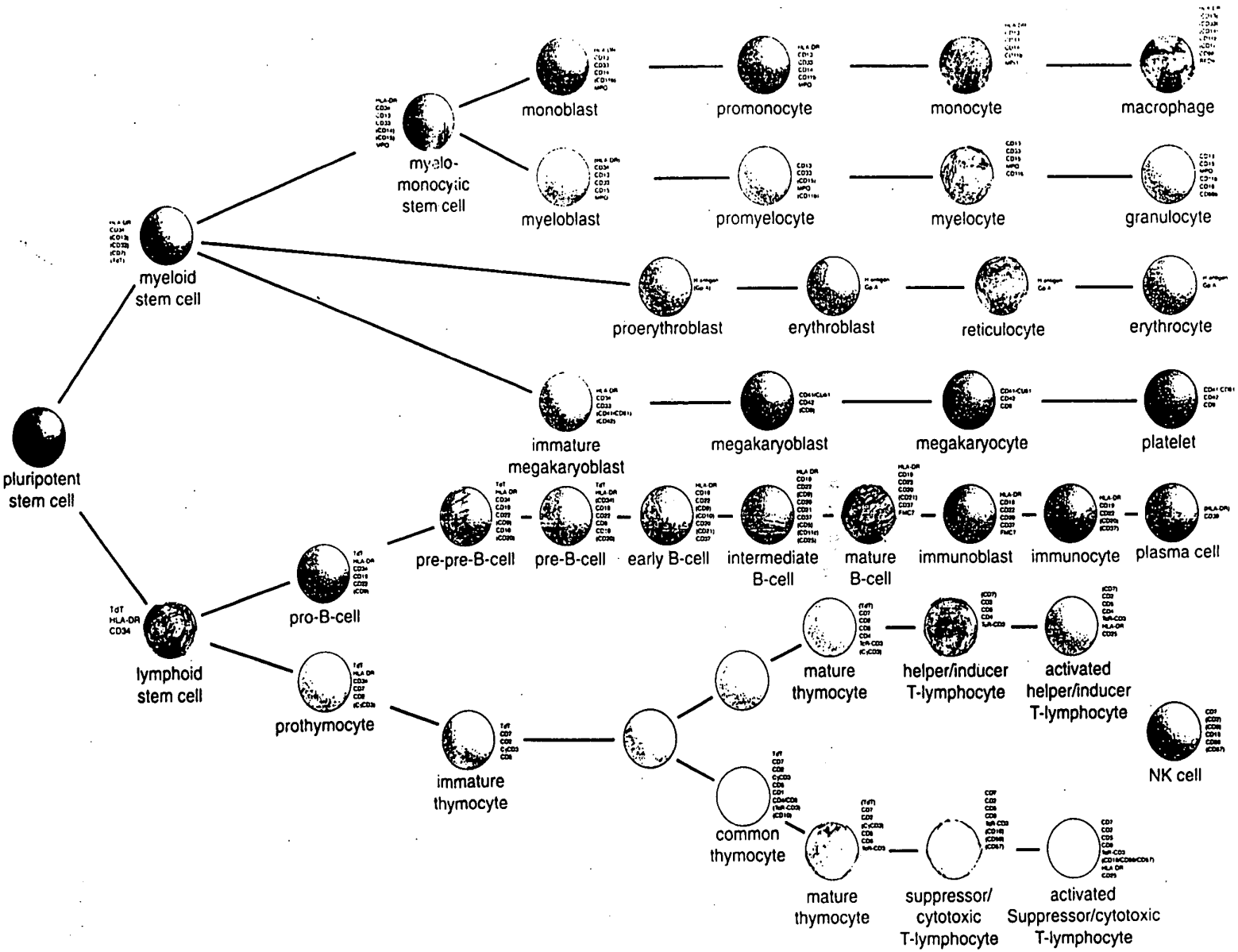
monoblast
promonocyte
monocyte
macrophage
myelo-
monocytic
stem cell
myeloblast
promyelocyte
myelocyte
granulocyte
myeloid
stem cell
proerythroblast
erythroblast
reticulocyte
erythrocyte
immature
megakaryoblast
megakaryoblast
megakaryocyte
platelet
pluripotent
stem cell
pre-pre-B-cell
pre-B-cell
early B-cell
intermediate
B-cell
mature
B-cell
immunoblast
immunocyte
plasma cell
pro-B-cell
lymphoid
stem cell
mature
thymocyte
helper/inducer
T-lymphocyte
activated
helper/inducer
T-lymphocyte
prothymocyte
NK cell
immature
thymocyte
common
thymocyte
mature
thymocyte
suppressor/
cytotoxic
T-lymphocyte
activated
Suppressor/cytotoxic
T-lymphocyte

FIGURE 2